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The intellectual disability risk gene Kdm5b regulates long term memory consolidation in the hippocampus

The intellectual disability risk gene *Kdm5b* regulates long term memory consolidation in the hippocampus

Leticia Perez-Sisques[1,2], Shail Bhatt[1], Rugile Matuleviciute[2], Talia Gileadi[1], Eniko Kramar[3], Andrew Graham[1], Franklin G. Garcia[3], Ashley Keiser[3], Dina P. Matheos[3], James A. Cain[1], Alan M. Pittman[4], Laura C. Andreae[2], Cathy Fernandes[2,5], Marcelo A. Wood[3], K. Peter Giese[6] and M. Albert Basson[1,2,7]

[1] Centre for Craniofacial and Regenerative Biology, Guy's Hospital, King's College London, London SE1 9RT, UK
[2] MRC Centre for Neurodevelopmental Disorders, Institute of Psychiatry, Psychology and Neuroscience, King's College London, New Hunt's House, London SE1 1UL, UK
[3] Department of Neurobiology and Behavior, School of Biological Sciences, University of California Irvine, Irvine, CA, USA
[4] St. George's University of London, Cranmer Terrace, London SW17 0RE, UK
[5] Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, 16 De Crespigny Park, London SE5 8AB, UK
[6] Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Rd, London SE5 9RT, UK
[7] Department of Clinical and Biomedical Sciences, Faculty of Health and Life Sciences, Hatherley Laboratories, Prince of Wales Road, University of Exeter Medical School, University of Exeter, Exeter, EX4 4PS, UK

Correspondence:

Prof. M. Albert Basson
Department of Clinical and Biomedical Sciences
University of Exeter Medical School
Hatherley Laboratories
Prince of Wales Road
Exeter
EX4 4PS
UK

Abstract

The histone lysine demethylase KDM5B is implicated in recessive intellectual disability disorders and heterozygous, protein truncating variants in *KDM5B* are associated with reduced cognitive function in the population. The KDM5 family of lysine demethylases has developmental and homeostatic functions in the brain, some of which appear to be independent of lysine demethylase activity. To determine the functions of KDM5B in hippocampus-dependent learning and memory, we first studied male and female mice homozygous for a *Kdm5b$^{\Delta ARID}$* allele that lacks demethylase activity. *Kdm5b$^{\Delta ARID/\Delta ARID}$* mice exhibited hyperactivity and long-term memory deficits in hippocampus-dependent learning tasks. The expression of immediate early, activity-dependent genes was downregulated in these mice and hyperactivated upon learning stimulus compared to wildtype mice. A number of other learning-associated genes was also significantly dysregulated in the *Kdm5b$^{\Delta ARID/\Delta ARID}$* hippocampus. Next, we knocked down *Kdm5b* specifically in the adult, wildtype mouse hippocampus with shRNA. *Kdm5b* knockdown resulted in spontaneous seizures, hyperactivity and hippocampus-dependent long-term memory and long-term potentiation deficits. These findings identify KDM5B as a critical regulator of gene expression and synaptic plasticity in the adult hippocampus and suggest that at least some of the cognitive phenotypes associated with *KDM5B* gene variants are caused by direct effects on memory consolidation mechanisms.

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79 **Significance statement**

80 The histone lysine demethylase KDM5B has been implicated in cognitive performance and

81 intellectual disability conditions in the human population. In the present manuscript we show

82 that mice expressing a demethylase-deficient KDM5B and mice with a specific knockdown of

83 KDM5B in the adult hippocampus exhibit hippocampus-dependent learning and memory

84 phenotypes. Molecular analyses suggest a key role for KDM5B in regulating the dynamic

85 expression of activity-regulated genes during memory consolidation. Deficits in LTP are

86 present in mice with KDM5B knockdown. Together, these findings provide the first evidence

87 for a direct function for KDM5B in memory consolidation in the hippocampus.

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Introduction

Genome sequencing studies have implicated genes encoding chromatin modifying factors in intellectual disability and autism spectrum disorders (ID and ASD). These include the gene KDM5B (Lysine demethylase 5B), recessive mutations of which are linked to a rare ID syndrome (Faundes et al., 2018; Martin et al., 2018). Intriguingly, *KDM5B* is tolerant to loss-of-function mutations, and a significant proportion of such heterozygous *KDM5B* variants are incompletely penetrant and inherited (Wang et al., 2022; Zhou et al., 2022). A recent study reported that rare protein truncating variants in *KDM5B* are associated with large effects on cognitive function in the population (Chen et al., 2023). Specifically, protein truncating variants in *KDM5B* negatively correlated with educational attainment, reaction time and verbal-numerical reasoning. This study also reported an association of protein truncating variants of *KDM5B* with neurodevelopmental and psychiatric disorders and epilepsy. The same study found initial evidence that a heterozygous and homozygous deletion of *Kdm5b* exon 7 in mice was associated with dose-dependent cognitive deficits and increased anxiety (Chen et al., 2023). Previous studies have reported significant embryonic or perinatal lethality of homozygous *Kdm5b* loss-of-function mutations in mice. The deletion of exon 1 was associated with lethality by embryonic day 7.5 (Catchpole et al., 2011), whereas either exon 6 (Albert et al., 2013) or exon 7 (Martin et al., 2018) deletion was associated with 36-44% postnatal viability, suggesting that the deletion of exons 6 or 7 produces hypomorphic alleles with partially penetrant effects.

KDM5B, together with KDM5A and KDM5C, are the only histone lysine demethylases known to demethylate trimethylated lysine 4 on histone 3 (H3K4me3), a post-translational histone modification typically present at active gene promoters. H3K4me3 can recruit components of transcriptional initiator (Vermeulen and Timmers, 2010; Lauberth et al., 2013), and integrator complexes (Wang et al., 2023). The latter is associated with the regulation of transcriptional output by stimulating transcriptional elongation (Wang et al., 2023). As KDM5B specifically

117 removes H3K4me3 to counteract this process, the lack of KDM5B demethylase activity is

118 predicted to lead to transcriptional de-repression of direct target genes. H3K4me3 is also

119 enriched at non-methylated CpG island promoters, where it is thought to counteract DNA

120 methylation (Hughes et al., 2020).

121

122 The *KDM5A-D* genes in mammals are paralogues of the ancestral *KDM5* gene in

123 *Drosophila. KDM5* mutation is also associated with lethality (Gildea et al., 2000). *Drosophila*

124 *KDM5* can regulate gene expression with multiple mechanisms, including a mechanism that

125 requires the PHD domain of the protein, and appear to function independently from the

126 demethylase activity of the protein (Liu and Secombe, 2015). Indeed, *Drosophila* KDM5 and

127 mammalian KDM5 proteins interact with other chromatin factors and complexes that function

128 as transcriptional repressors, including components of Sin3/HDAC1 and NuRD complexes

129 (Yheskel et al., 2023). Thus, some KDM5B-mediated transcriptional repressionmay be

130 mediated by demethylase-independent mechanisms. Flies with mutations in the

131 demethylase domains of the protein survive, consistent with demethylase-independent

132 functions, but exhibit specific learning phenotypes (Zamurrad et al., 2018; Belalcazar et al.,

133 2021).

134

135 Mutations in the lysine methyltransferases responsible for H3K4me3, KMT2A and KMT2B

136 are also associated with autosomal dominant ID syndromes (Jones et al., 2012; Faundes et

137 al., 2018). Animal experiments have shown that a learning stimulus results in a rapid and

138 transient increase in H3K4me3 levels in the hippocampus, similar to immediate early gene

139 induction (Gupta et al., 2010). Consistent with a functional role for H3K4me3, the lysine

140 methyltransferases, KMT2A and KMT2B are necessary for normal hippocampus-dependent

141 learning and memory in mice (Kerimoglu et al., 2013; Kerimoglu et al., 2017).

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143   Together, these studies suggest that dysregulation of H3K4me3, either by loss of function of

144   methyltransferases or demethylases, can result in ID. Conditional deletion of *Kmt2a* and

145   *Kmt2b* genes in post-mitotic neurons in the mouse, was sufficient to cause learning and

146   memory phenotypes, suggesting that defects in the H3K4me3 machinery directly impacts

147   memory consolidation mechanisms in the hippocampus (Kerimoglu et al., 2013; Kerimoglu

148   et al., 2017). Thus, to understand how KDM5B loss-of-function affects learning and memory,

149   we set out to determine if KDM5B has a direct function in memory consolidation, as opposed

150   to having exclusively developmental functions. We focused on the hippocampus as the

151   function of this brain region in learning and memory is well-established, and hippocampus-

152   dependent memory tasks and electrophysiological correlates such as long-term potentiation

153   (LTP) are robust in mice.

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157   **Materials and Methods**

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159   **Animals**

160   Experiments with mice carrying the *Kdm5b*^{tm1Jtpu}, referred to as the *Kdm5b*^{ΔARID} allele

160   Experiments with mice carrying the $Kdm5b^{tm1Jtpu}$, referred to as the $Kdm5b^{\Delta ARID}$ allele

161   (Catchpole et al., 2011) were performed at King's College London. $Kdm5b^{\Delta ARID/\Delta ARID}$ males

162   on a C57BL/6J background were crossed with 129S2/Sv females to produce heterozygous

163   F1 mice, that were then inter-crossed to produce WT and homozygous $Kdm5b^{\Delta/\Delta}$ F2 mice for

164   experiments. Experimental cohorts of mice were derived from 5 (studies with naïve mice) to

165   15 (for behavioural analyses) litters. Animals were housed in ventilated cages (37x20x16cm)

166   with *ad libitum* access to water and food (Labdiet Picolab rodent irradiated diet, #5R53) and

167   kept at 19–22°C and 40–60% humidity, under a 12:12-h light/dark cycle. The cages

168   contained bedding (Lignocel wood fiber) and nesting. A maximum of five animals were

169   housed in the same cage. All animal procedures were approved by the King's College

170   London AWERB and the UK Home Office.

171   **Genotyping of mice**

172   Genotyping was performed by extracting genomic DNA from ear notches as previously

173   described (Hurley et al., 2021). The following primer pairs to detect the WT and the mutant

174   alleles were used: WT forward 5'- CCTTAGACGCAGACAGCACA-3', WT reverse 5'-

175   CGTGTTTGGGCCTAAATGTC-3', Kdm5b-ΔARID forward 5'-

176   TGCTCCTGCCGAGAAAGTATCC-3' and Kdm5b-ΔARID reverse 5'-

177   CCACCCCCCAGAATAGAATGA-3'. Thermal cycles for the genotyping reactions were as

178   follows: 95°C, 2 min; 35x (95°C, 15 sec; 64°C, 15 sec; 72°C, 15 sec); 72°C, 12min.

179   ***Kdm5b* knock-down**

180   Adult (8-12 week old) female C57BL/6J mice received 1 μl (1×10^{10} viral particles) of either

181   AAV1-CMV-GFP-U6-mKdm5b-shRNA, Vector Biolabs #shAAV-262769) or control AAV1-

182   CMV-GFP-U6-scrambled shRNA, #7040) in both dorsal hippocampi as described (Kwapis et

183 al., 2018). These experiments were approved by the UCI IACUC and KCL AWERB and UK

184 Home office.

Western blot

186 Brain cortices were dissected from adult mice and whole cell protein prepared by lysing in

187 8M urea, 1% CHAPS, 50mM Tris (pH 7.9) lysis buffer containing protease inhibitors.

188 Samples were rotated for 30 min at 4°C and then centrifuged for 50 min to remove DNA.

189 Supernatant was stored at -80°C. All reagents and machinery were obtained from BioRad

190 unless stated otherwise. Samples were prepared with Laemmli buffer containing 10% β-

191 mercaptoethanol and resolved with 7.5% Mini-PROTEAN pre-cast polyacrylamide gels and

192 Tris/Glycine/SDS buffer. 60μg of protein were prepared to detect KDM5B protein in brain

193 lysates. Proteins were transferred to a nitrocellulose membrane with the TransBlot turbo

194 system (High molecular weight transfer program). Membranes were blocked with 5% BSA

195 (Sigma) diluted in tris-buffered saline containing 0.1% Tween-20 (TBS-T). Primary

196 antibodies were diluted in TBS-T containing 5% BSA. Primary antibodies were incubated

197 overnight at 4°C in 5% BSA in TBS-T. Membranes were incubated with secondary

198 antibodies diluted in TBS-T containing 5% BSA for 1h at room temperature. Proteins were

199 detected with Clarity ECL reagent and membranes were imaged using the Chemidoc

200 system. Densitometric analyses were performed with ImageJ software (NIH). The following

201 antibodies were used: anti-KDM5B (Abcam, ab181089, 1:1000), αTubulin (Upstate, 05-829),

202 goat anti-rabbit and anti-mouse HRP secondary antibodies (Thermo Fisher Scientific,

203 #31460 and Proteintech, #SA00001-1, respectively, 1:5000). Uncropped full scans of the

204 blots shown in Figure 1B are shown in Extended Data Figure 1-1.

Immunofluorescence

206 Animals were perfused with either 1x PBS or 1x PBS followed by 4% paraformaldehyde

207 (PFA). Brains were removed and fixed overnight in 4% PFA. Samples were cryopreserved

208 with a sucrose gradient (5%, 15%, 30%, one day each). Free-floating coronal brain sections

209 (20-30μm) were obtained with a cryostat. Sections were first washed twice in PBS-T (1x

210 PBS 0.3% Triton X-100) and blocked for 1h in blocking buffer (BB: PBS-T 3% NGS 3%

211 BSA). Following blocking, primary antibodies were incubated overnight in BB at 4°C. The

212 following day, slices were washed twice with PBS-T and secondary antibodies were

213 incubated in BB for 2h. Samples were washed three times with PBS-T before nuclei staining

214 with Hoechst3332 (1:5000). Two final washes with 1x PBS were performed before mounting

215 with anti-fade mounting media (Abcam, ab104135). The following antibodies were used

216 (1:200 dilution): Npas4 (Activity signaling, NP41-2), GFP (Abcam, ab13970), H3K4me3 (Cell

217 Signaling Technologies, 9751S), goat anti-rabbit 488 (Thermo Fisher Scientific, A11031),

218 and goat anti-chicken 488 (Thermo Fisher Scientific, A11039. Images were acquired with a

219 Zeiss Axio Observer7 microscope (20x magnification) and Zen Blue software. Image

220 acquisition and analysis were performed blindly. The number of Npas4-positive nuclei in the

221 hippocampal layers CA3 and DG was quantified manually with the Cell Counter plug-in on

222 ImageJ-FIFI (NIH). The number/density and mean staining intensity/cell of H3K4me3 were

223 analysed in CA1/2, CA3 and dentate gyrus with QuPath (Bankhead et al., 2017). The

224 following settings were used to detect the positive cells: Requested pixel size: 0.5;

225 Background radius: 8; Median filter radius: 0; Sigma: 1.5; Cell expansion: 2 (all in μm);

226 Minimum area: 10; Maximum area: 400 (in μm^2); Threshold: 10.

227 **Golgi staining and spine analysis**

228 Fresh brain hemispheres were processed with the FD Rapid GolgiStain^{TM} kit (FD

229 Neurotechnologies) following manufacturer's instructions. Briefly, 100 μm sections were

230 obtained with a vibratome and mounted on gelatine-coated superfrost slides. Following the

231 staining procedure, brightfield images of impregnated dendrites from dorsal hippocampal

232 neurons were captured with a Zeiss Axio Observer7 microscope (63x magnification) and Zen

233 Blue software. Stacks were taken every 0.2 μm and analysed manually with FIJI. Spine

234 density was calculated in 10 proximal dendrites per area and animal, starting 5 μm apart

235 from the ramification. Spine density values are shown as average for each area and animal.

Behaviour

Different cohorts of control and *Kdm5b*-mutant mice were used for the experiments in this study: 1) battery of behavioural tests (Fig. 3), 2) confirmation of the anxiety-related phenotype, 3) RNAseq with naïve animals (Fig. 4A,B), 4) confirmation of the RNAseq results by immunofluorescence and RT-qPCR (Fig. 4C-E), and 5) RNAseq to study gene expression changes upon learning (Fig. 5). For the *Kdm5b* knock-down experiment (Fig. 6), a cohort of mice was used for the behavioural and the electrophysiology analyses, and extra cohorts were used to confirm the *Kdm5b* knock-down one, three, and seven weeks after the surgery. A final cohort was dedicated to study the effect of the knock-down on early gene expression 11 days after the surgery.

Behavioural assessments of F2 mice (cohort 1) started at 7-8 weeks of age, consisting of the following: handling, open field test, object location memory test, elevated plus maze, spontaneous alternation in a Y-maze test, Morris water maze, fear conditioning test and measurement of grip strength. Mice were left to rest for at least 2 days in between tests. Mice in cohorts 3, 4 and 5 were 7-8 weeks old when samples were collected.

Behavioural experiments were conducted between 8:00 and 18:30 under standard room lighting conditions unless stated otherwise. Cages were changed every two weeks but never less than 48h before the day of testing. Behaviours were tracked using Ethovision (Noldus Information Technologies bv, Wageningen, The Netherlands). After each trial of a specific test, boli and urine were removed, and the test area was cleaned with 1% Anistel® (high level surface disinfectant, Trisel Solution Ltd, Cambridgeshire, UK, for cohort 2) or 10% ethanol (cohort 1) to remove any odours. The experiments were blinded and randomized by blocks of mice. Littermates were used as controls with multiple litters examined per experiment (15 for cohort 1 and 12 for cohort 2). Mice were habituated to the behavioural room conditions for at least 30 minutes before the start of each testing session. Before the start of the experiments, mice were individually handled for 5 consecutive days, 2 minutes each day.

263 Behavioural assessments of shRNA mice were started 2-3 weeks after stereotactic

264 surgeries. Mice were first tested in the elevated plus maze to determine effects on activity

265 and anxiety, handled for 5 days as described and habituated to the test arena for 6 days

266 before training with two identical objects for 10 minutes, followed by a 5 min test session 24h

267 later with one of the objects moved to a new location (Kwapis et al., 2018). Two weeks later,

268 tissue was collected for acute slice preparations and LTP.

269 *Open field*

270 The circular open field arena was made of clear acrylic with a grey base, with internal

271 dimensions of 40 cm diameter and 40 cm high as described (Whittaker et al., 2017). Light

272 intensity inside the arena was 10 lux. Two virtual areas were drawn on Ethovision: a centre

273 zone, 20cm diameter, and a ring 5 cm thick around the perimeter of the arena was defined

274 as the outer zone. Test mice were placed in the open field facing an outer wall to begin the

275 test. Its locomotor activity was tracked for 5 minutes. Total distance moved (cm) in the outer

276 zone was used as a measure of the level of locomotive activity and time (s) spent in the

277 centre zone used as a measure of anxiety.

278 *Object location memory test*

279 The test was performed as described in (Vogel-Ciernia et al., 2013). The arena (40x40x40

280 cm) was made of white acrylic and had a black stripe in one of the walls as an internal cue.

281 Light intensity was 40 lux in the centre of the arena. Briefly, mice were habituated to the

282 arena for 6 consecutive days, 5 minutes/day. During the training session, mice were placed

283 for 10 minutes in the arena with the two identical objects (100 ml glass beakers filled with

284 cement to prevent them being moved by the mice) next to the wall with the visual cue. 24 h

285 later, during the test session, one of the two objects was randomly moved to the opposite

286 side of the arena, in a centre position, and mice were left to explore the arena for 5 minutes.

287 Exploration was manually scored and the following criteria was used: nose of the mouse is

288 within 1cm of the object, with their head directly facing it. Rearing, standing on the object,

and digging near it were not considered as exploratory behaviours. Discrimination index (DI) was calculated as: (time exploring new location – time exploring old location) / (total exploration time) * 100. Exclusion criteria included exploration time below 3 seconds for either training or testing sessions, and DI above +/-20 for the training session.

Elevated plus maze (EPM)

The EPM was made of black acrylic and consisted of four arms (30 x 5 cm). The two opposing closed arms were enclosed by 15 cm high walls on each side and ends. The two opposing open arms were open, as well as the centre platform (5 x 5 cm). The maze was elevated 40 cm above ground. Light intensity was 100 lux on the open arms and 10 lux on the closed arms. The number of entries onto, time spent on, and latency to enter the closed and open arms were manually scored. An arm entry is defined when all 4 paws were located inside the arm. Mice were placed in the centre platform of the EPM facing a closed arm to start the 5 minutes test.

Spontaneous alternation in a Y-maze

The Y maze apparatus was made of grey acrylic and consisted of three arms, 120° from each other. All arms were 45cm long, 5 cm wide and enclosed by a 10cm wall. Extramaze visual cues were placed at the end of each arm. One arm was considered as the central arm, and the other two arms were randomly closed during the training session. During the training session, mice were placed in the maze in the central arm and allowed to explore it for 10 minutes, after which they were returned to their homecage. After 1h, the testing session was conducted. Both arms were open, and mice were placed in the central arm and allowed to freely explore the maze for 5 minutes. The first choice to turn either to the familiar arm or the new arm (percentage of alternation rate) was monitored, when all four paws were inside that arm. Arm preference was automatically monitored with Ethovision and the Discrimination index was calculated as (time exploring new arm – time exploring old arm) / (total exploration time of the old and new arms) * 100.

Morris water maze

Spatial learning and memory were assessed using a Morris water maze (MWM). The maze (1 m diameter) was filled with water, made opaque with a non-toxic white aqueous emulsion. Water temperature was 23°C, and light intensity was 100lux. A set of extra-maze visual cues were suspended around the pool. Four alternative start positions were nominated to provide the virtual division of the tank into four quadrants. Mice were trained to find a hidden platform (10 cm diameter), submerged 2 cm below the water surface, for 8 consecutive days, four trials a day. Once they found the platform, mice were left on it for 15 seconds before returning them to their homecage. Mice that failed to locate the hidden platform after 60 seconds were placed on the platform for 15 seconds. Animals that failed to stay on the platform following two days of training were excluded from the study. On probe trial the platform was removed and mice were placed into the pool for 60 seconds so they could freely explore the tank. To check for deficiencies in vision or locomotion, mice were placed in the pool with a visible platform (1cm above the surface, with a flag attached to it), one day, four trials. Mouse movement was tracked with Ethovision to calculate mean speed, total distance travelled in the pool and in each quadrant, and the number of platform crossings during the probe trial.

Fear conditioning test

Animals were placed in a soundproof fear conditioning apparatus with stainless steel metal grid floor, containing a camera (Med Associates Inc.). To provide an olfactory cue, an ethanol-soaked tissue was placed under the grid in both training and testing sessions. Mouse behaviour was recorded with VideoFreeze software. For conditioning, mice were placed inside the chamber and left to freely explore it. After 148 seconds, three electric shocks (0.7mA, 2 sec each, 30 seconds apart) were administered. The animals were then removed from the testing chamber after 30 additional seconds. 24 h later, mice were placed in the same chamber for 5 minutes. Freezing behaviour was manually scored from recorded

videos during training and testing sessions. Freezing behaviour was defined as the complete

lack of movement during the first 2 seconds of each 5-second window.

Grip strength measurements

To assess the neuromuscular ability of the animals, fore- and hindlimb grip strength was

measured with a Linton Grip Strength Meter (MJS Technology, Ltd). Mice were pulled across

the meter from left to right measuring forelimb and then hindlimb strength. The average of

three measurements per limb and mouse was taken.

RNA extraction

Total RNA was extracted from hippocampal samples with 1ml TRIzol (Thermo Fisher

Scientific) and further purified with the Monarch Total RNA miniprep kit (New England

Biolabs) following the manufacturer's recommendations (including DNAse treatment step).

qRT-PCR analysis

cDNA was synthesised from 200-400ng RNA with UltraScript 2.0 cDNA Synthesis kit (PCR

Biosystems) according to the manufacturer's instructions. qRT-PCRs were performed on a

BioRad CFX384 using qPCRBIO SyGreen Mix Lo-ROX (PCR Biosystems). Relative

expression levels were calculated using the $2^{-\Delta\Delta CT}$method and *Hprt* or *Gapdh* were used as

endogenous control genes. For shRNA experiments, the PrimeTime probe-based gene

expression system (IDT) was used to quantify *Kdm5b* expression, relative to *Hprt*. Primer

sequences: Arc (Fw: 5'-CTCAACTTCCGGGGATGCAG-3', Rv: 5'-CTGGTATGAATCACTGGGGGC-3'), cFos (Fw: 5'-AGAGCGGGAATGGTGAAGAC-3', Rv: 5'-AGTTGATCTGTCTCCGCTTGG-3'), Egr1 (Fw: 5'-TGAGCACCTGACCACAGAGTC-3', Rv: 5'-TAACTCGTCTCCACCATCGC-3'), Egr2 (Fw:5'-GTGCTGCCTGACAGCCTCTA-3', Rv: 5'-TTGATCATGCCATCTCCCGCC-3'), Gapdh (Fw: 5'-AGGTCGGTGTAACGGATTTG-3', Rv: 5'-TGTAGACCATGTAGTTGAGGTCA-3'), Hprt (Fw: 5'-GTCCCAGCGTCGTGATTAGC-3', Rv: 5'-TGGCCTCCCATCTCCTTCAT-3'), Kdm5b (Fw: 5'-AAGCCAAGCTCTGTTCAGCAA-3', Rv: 5'-GAAGGCAATCGTTCTTCTCACT-3'), Npas4

367 (Fw: 5'-CTGCATCTACACTCGCAAGG-3', Rv: 5'-GCCACAATGTCTTCAAGCTCT-3'),

368 Kdm5b (for Fig. 6, Fw: 5'-CAAGAGCCCACTGAGAAGAAA-3', Rv: 5'-

369 TCCACATAAGAGGCACACATAC-3') and Hprt (for Fig. 6, Fw: 5'-

370 TGCTCGAGATGTCATGAAGG-3', Rv: 5'-CTTTTATGTCCCCCGTTGAC-3').

371 **RNAseq**

372 Total RNA (n=4/genotype, balanced for sex) were sent to Novogene for library preparation

373 and sequencing. After mRNA enrichment, mRNA quality was analysed using Agilent Total

374 RNA 6000 Pico on a Bioanalyser (Agilent, 2100). Pair-end sequencing (150bp read length)

375 was performed on the Illumina Novaseq 6000 platform. Further data analyses were

376 performed using the Galaxy Europe server (https://usegalaxy.eu)

377 (https://academic.oup.com/nar/article/50/W1/W345/6572001). Quality of the raw data was

378 checked using FastQC (v0.11.9). Reads were aligned to the mouse genome (mm10) using

379 RNA STAR (v2.7.8a), and aligned reads were counted using FeatureCounts (v2.0.1).

380 Differential expression analyses were performed using DESeq2 (v2.11.40.7). Multiple

381 comparisons were controlled for using an FDR < 0.05. Exact p values and adjusted p-values

382 for all differentially expressed genes are listed in Extended data Table 4-1 and Table 5-1.

383 Genes with adjusted p-value <0.05 were considered as differentially expressed genes

384 (DEGs). Heatmaps were generated with the R package pheatmap. Volcano plots were

385 generated in GraphPad Prism 9.4.1. Differentially expressed genes in at least one

386 comparison (any timepoint between genotypes, or 1h/0h, 3h/0h and 3h/1h within the same

387 genotype) were clusterised with k-means clustering and selecting the optimal cluster number

388 for the dataset (k=4). Activity-induced genes were obtained from (Chen et al., 2017), with

389 only differentially upregulated transcripts detected by both RNAseq and TRAPseq included.

390 Gene ontology analyses were conducted using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost),

391 where GO molecular function and GO biological process of size 0-1500 were checked. The

392 applied threshold was "Benjamini Hochberg FDR < 0.05".

393

Electrophysiology

Female mice (n=4/treatment, 8 total) were sacrificed for electrophysiology and hippocampal slices prepared as described previously (Vogel-Ciernia et al., 2013). Mice were anesthetized, decapitated, and the brains rapidly removed into ice-cold, oxygenated dissection medium containing (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 5 MgSO4, 0 CaCl2, 26 NaHCO3, and 10 glucose. Hippocampal slices (320 μm, coronal) were cut from a vibratome (Leica, Model:VT1000S) before transferred to an interface recording containing prewarmed (31 ± 1^0 C) artificial cerebrospinal fluid (aCSF) composed of (in mM): 124 NaCl, 3 KCl, 1.25 KH2PO4, 1.5 MgSO4, 2.5 CaCl2, 26 NaHCO3, and 10 glucose. Slices were perfused continuously at a rate of 1.75-2 ml/min while the surface of the slices were exposed to warm, humidified 95% O2/5% CO2. Recordings began following at least 2h of incubation.

Field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1b stratum radiatum apical dendrites using a glass pipette filled with 2M NaCl (2-3 MΩ) in response to orthodromic stimulation (twisted nichrome wire, 65 μm diameter) of Schaffer collateral-commissural projections in CA1c stratum radiatum. Pulses were administered 0.05 Hz using a current that elicited a 50% maximal spike-free response. After maintaining a stable baseline (20 min), long-term potentiation (LTP) was induced by delivering 5 'theta' bursts, with each burst consisting of four pulses at 100 Hz separated by 200 msec (i.e., theta burst stimulation or TBS). The stimulation intensity was not increased during TBS. Data were collected and digitized by NAC 3.0 (Neurodata Acquisition System, Theta Burst Corp., Irvine, CA) and stored on a disk.

Data in the text are presented as means ± SD, while in the figures as mean ± SEM. The fEPSP slope was measured at 10–90% fall of the slope and data in figures on LTP were normalized to the last 20 min of baseline.

Statistics

419   Data are reported as mean ± SEM. Graphs show individual data points. Normal distribution

420   was tested with d'Agostino and Pearson omnibus, Shapiro-Wilk and Kolmogorov-Smirnov

421   normality tests. If the test was passed, statistical analysis was performed using parametric

422   statistical analyses. Before pairs of comparisons, we performed the F test to compare

423   variances. In experiments with normal distribution statistical analyses were performed using

424   the unpaired two-sided Student's t test. T-test with Welch's correction was applied when

425   variances were unequal. Two- and Three-way ANOVA with the appropriate post hoc tests

426   were also performed as indicated in the figure legends. Significant p values (p<0.05) are

427   reported in the results section and/or figure legends provide details of relevant statistical

428   parameters, including group sizes. Statistical analyses were performed with GraphPad Prism

429   (version 9.4.1). Experiments in this study were blinded and animals randomised for in vivo

430   studies.

431

432   **Results**

433   **KDM5B demethylase-deficient mice exhibit megalencephaly**

434    $Kdm5b^{\Delta ARID/\Delta ARID}$ ($Kdm5b^{\Delta/\Delta}$) mice were generated to circumvent the embryonic and early

435   postnatal lethality of other loss-of-function models. The deletion of exons 2 to 4 leads to a

436   partial truncation of the JmjN domain and the deletion of the ARID domain of the KDM5B

437   protein (Fig. 1A), that disrupts its H3K4me3 demethylase activity (Jamshidi et al., 2021). We

438   confirmed the loss of full-length KDM5B protein and the appearance of a shorter KDM5B-

439   ΔARID protein in the brain of adult $Kdm5b^{\Delta/\Delta}$ mice by Western blot (Fig. 1B,C). The mutant

440   KDM5B-ΔARID protein appears to be either unstable or not as well detected by KDM5B-

441   specific antiserum in western blot, leading to an apparent reduction in total KDM5B protein

442   levels in the homozygous mutants, compared to wildtype mice (Fig. 1D). We did not observe

443   differences between male and female animals.

444 To assess the impact on H3K4me3, hippocampal sections from $Kdm5b^{\Delta/\Delta}$ and control mice

445 were immunostained with an antibody to H3K4me3. We observed a clear increase in the

446 number of H3K4me3-positive cells in the hippocampus (Fig. 1E,F). This increase was

447 observed in both sexes (Two-way ANOVA sex effect: $F_{1,4}$=0.1824, p=0.6913). Moreover, the

448 intensity of H3K4me3 across the population of cells in the hippocampus shifted towards

449 increased H3K4me3 in the mutants in all hippocampal subregions, cornu ammonis 1 (CA1)

450 (Fig. 1G,H), cornu ammonis 3 (CA3) (Fig. 1I,J) and dentate gyrus (DG) (Fig. 1K,L).

451 In an attempt to further reduce any developmental abnormalities associated with KDM5B

452 deficiency, all animals used in this study were on a C57BL/6Jx129S2/Sv F2 genetic

453 background (Zou et al., 2014, and data from the International Mouse phenotyping

454 Consortium (IMPC): http://www.mousephenotype.org/). Homozygous $Kdm5b^{\Delta/\Delta}$ mice

455 generated on this genetic background generated from heterozygous intercrosses were

456 present at expected ratios at P21 (Fig. 2A). Postnatal $Kdm5b^{\Delta/\Delta}$ mice displayed a growth

457 deficit and reduced body weights (Fig. 2B). Homozygous mutants exhibited increased brain-

458 to-body weight ratios at postnatal day 21, while the liver-to-body weight ratios did not differ

459 between genotypes, suggesting specific neurodevelopmental alterations (Fig. 2C-G).

460 Histological assessments did not reveal any gross abnormalities in cortical architecture (Fig.

461 2H,I) or spine density in proximal dendrites of CA1) pyramidal neurons and of DG granule

462 cells in the hippocampus (Fig. 2J).

463

464 **Hippocampus-dependent learning and memory deficits in $Kdm5b^{\Delta/\Delta}$ mice**

465 To determine whether KDM5B loss of function affects learning and memory, we tested these

466 mice in hippocampus-dependent tasks. Data from males and females were combined,

467 unless mice of different sexes exhibited differences in specific tests, where the data from

468 both sexes are shown. During the handling sessions we observed that the reduced body

469 weight observed at pre-weaning stages was still evident at 2 months of age (Fig. 3A).

470 *Kdm5b$^{\Delta/\Delta}$* mice exhibited hyperactivity in the open field (Fig. 3B). They also spent less time in

471 the inner area of the arena, suggesting an anxiety-like phenotype (Fig. 3C). However, this

472 phenotype was not observed in the elevated plus maze (EPM) in the same cohort of mice

473 (Fig. 3D) and was not consistent in a second cohort (for that cohort: distance travelled in the

474 open field genotype effect: p=0.9966; time (%) spent in the open arms in the EPM genotype

475 effect: *p=0.0385), suggesting that the anxiety-like phenotype is variable and potentially

476 secondary to hyperactivity. Next, we performed the novel object location memory test (OLM)

477 to study long-term spatial memory. A significant deficit in long-term spatial memory was

478 observed in the mutant mice 24 h after training (Fig. 3E). Exploration times were equal

479 between genotypes in both training and testing sessions (Fig. 3F), indicating a specific deficit

480 in object location memory. In contrast, short term spatial memory and cognitive flexibility was

481 preserved in *Kdm5b$^{\Delta/\Delta}$* mice, as judged, respectively, by the discrimination index and

482 spontaneous alternation percentage in the spontaneous alternation test in a Y-maze (Y-SAT)

483 (Fig. 3G,H). Next, we examined long-term spatial learning and reference memory using the

484 Morris water maze (MWM). During the training sessions with the hidden platform version of

485 the test, performance improved significantly in both groups, although escape latency in

486 mutant mice remained significantly higher compared to WT littermates (Fig. 3I). During the

487 probe trial on day 8, *Kdm5b$^{\Delta/\Delta}$* mice displayed a reduced number of platform crossings (Fig.

488 3J), indicating impaired spatial memory. All groups of mice explored the target quadrant

489 significantly more than other quadrants during the probe trial, indicating that spatial memory

490 had been acquired, but homozygous females showed significantly reduced exploration time

491 in the target quadrant compared to wildtype females (Fig. 3K), consistent with a deficit in

492 spatial learning. There was no effect on swim speed during the Morris water maze test,

493 excluding a reduction in the ability of mice to explore the water maze as a potential

494 explanation for the reduced performance of the mutants (Fig. 3L). No significant differences

495 were observed between genotypes in the visible platform task in male mice, excluding poor

496 vision, altered motivation or sensorimotor alterations as a cause for the deficit in mutant

497 male mice. However, an increased latency to find the visible platform was observed in

females (Fig. 3M), which might be indicative of visual or motivation changes, or subtle anxiety differences that could be contributing to the deficits observed during the training and testing sessions. Grip strength analysis revealed reduced front- and hindlimb strength in mutant mice (Fig. 3N,O). Finally, we performed the contextual fear conditioning test to assess associative long-term memory. During the training session, both groups of mice increased their freezing behaviour following three unconditioned stimuli (US) of 0.7 mA, 2 s foot shocks) (Fig. 3P). However, freezing behaviour was significantly reduced in $Kdm5b^{\Delta/\Delta}$ mice compared to their wildtype littermates. In the testing session 24 h later, reduced freezing was observed on the mutant animals (Fig. 3Q). The increased movement during the training session was also observed when tracking the animals' movement, but their mean speed was similar during the shock administration, excluding differences in the ability of mutants to discern or respond to the shock (Fig. 3R). Nevertheless, the hyperactive phenotype in these mice (see Fig. 3B) should be considered as an inevitable confounding factor on this test. In summary, although the hyperactivity of the mutants complicates the interpretation of the contextual fear conditioning test, and confounds exist for the Morris water maze, $Kdm5b^{\Delta/\Delta}$ mice exhibited clear deficits in the hippocampus-dependent object location task, with no confounds apparent, leading us to conclude that these mice exhibit hippocampus-dependent learning and memory deficits

Altered baseline and learning-induced hippocampal gene expression in $Kdm5b^{\Delta/\Delta}$ mice

As KDM5B regulates gene transcription, we next asked if reduced KDM5B demethylase activity affected the transcriptional landscape in the mouse hippocampus. We performed RNA sequencing of adult wild-type and homozygous hippocampi. We detected 20 differentially expressed genes (DEGs), of which 14 were up- and 6 were down-regulated (Fig. 4A, B and Extended data Table 4-1). Intriguingly, two key early response genes associated with learning and memory, *Egr1* and *Npas4*, were amongst the downregulated

525 genes (Fig. 4B). To validate this finding, we quantified transcripts for several immediate early

526 genes (*Egr1*, *Npas4*, *cFos*, *Egr2* and *Arc*) by quantitative PCRs (qRT-PCR) from a separate

527 set of hippocampal samples. Consistent with the RNA-seq data, the expression of *Egr1*,

528 *Npas4* and *cFos* were significantly downregulated in the mutants (Fig. 4B). We next asked if

529 these results translated into alterations in protein levels. As Npas4 is specifically and

530 selectively induced by neuronal activity in the CA3 and DG regions of the hippocampus

531 (Ramamoorthi et al., 2011), Npas4-positive cells were quantified by immunostaining. The

532 number of Npas4-positive cells was reduced in $Kdm5b^{\Delta/\Delta}$ mice compared to their wildtype

533 littermates (Fig. 4D,E).

534 Activity-dependent transcription factors such as Npas4 are crucial for memory formation

535 (Weng et al., 2018; Sun et al., 2020). Thus, we decided to test if KDM5B deficiency is

536 associated with abnormal activity-dependent gene regulation during hippocampus-

537 dependent learning. We compared the hippocampal transcriptomes of three groups of mice

538 with RNA-seq: naïve animals, and mice that were sacrificed 1h or 3h following training in the

539 contextual fear conditioning (CFC) paradigm (see Extended data Fig. 5-1 and Table 5-1). As

540 validation, we were again able to confirm the reduction of immediate early gene expression

541 by qRT-PCR in these samples (Extended data Fig. 5-1A). To understand the differences in

542 activity-induced transcional responses between wildtype and mutant mice, we clustered

543 the differentially expressed genes by k-means clustering according to their trajectories

544 (Extended data Table 5-2). Four different clusters were identified, referred to as Clusters 1-4

545 below. Cluster 1 exemplified a typical immediate early gene trajectory of rapid, transient

546 induction at 1h (Fig. 5A). These genes were functionally enriched for transcriptional

547 regulation (Extended data Fig. 5-1G), consistent with the fact that the majority of these

548 genes encode transcriptional factors like c-Fos, Fosb, Egr1, Egr2 and Npas4. In agreement

549 with our previous experiments (Fig. 4), this analysis revealed that Cluster 1 genes were

550 downregulated in naïve, control mutant animals, compared to wildtypes (Fig. 5A). These

551 genes were rapidly induced in mutants, to a larger extent than in wildtypes, such that they

552 were expressed at the same level as wildtype controls at both 1h and 3h post-CFC (Fig. 5A).

553 This observation was confirmed by qRT-PCR for the immediate early genes: *Egr2*, *cFos*,

554 *Egr1, Npas4* and *Arc* (Extended data Fig. 5-1B-F).

555

556 We detected three other clusters with different trajectories (Fig. 5B-D). Cluster 2 genes

557 showed an induction that gradually increased over time after the learning stimulus (Fig. 5B).

558 These genes included late response genes, some with known functions in neurogenesis and

559 synaptic plasticity e.g. *Fgfr1* (Zhao et al., 2007). Similar to Cluster 1, Cluster 2 genes were

560 downregulated in mutants at baseline, and reached similar expression levels to wildtype

561 controls by 3h (Fig. 5B). Cluster 3 included genes that were constitutively overexpressed in

562 homozygous mutants (Fig. 5C). This cluster was enriched for transcriptional regulation and

563 cation binding proteins and included genes encoding transcription factors such as Stat4 and

564 Stat6, as well as Zn finger proteins. These genes included *Cacna1i*, a gene encoding a

565 calcium channel subunit. Gain-of-function mutations in this gene associated with

566 neurodevelopmental disorders and epilepsy (El Ghaleb et al., 2021). Cluster 3 also included

567 *C1ql2* and *C1ql3*, encoding complement proteins strongly expressed in mossy fibres with a

568 crucial role in the control of synapse stability and number (Matsuda et al., 2016). Cluster 4

569 genes represented genes that were downregulated in response to a learning stimulus in both

570 wildtype and mutant hippocampi, with the downregulation more pronounced in mutants (Fig.

571 5D). This cluster was enriched for transcriptional and signalling regulators (Extended data

572 Fig. 5-1G). Interestingly, these included the *Pde10a* gene, known to enhance fear memories

573 in certain contexts e.g. (Guo et al., 2016), but also genes like *Hgf* known to promote learning

574 and memory, perhaps providing an explanation of learning deficits in our KDM5B mutant

575 mice (Kato et al., 2012). Cluster 4 also includes *Top1*, whose inhibition leads to reduced

576 transcription of genes associated with ASD and neurotransmission regulation (Mabb et al.,

577 2014), and *Arid1b*, which is also implicated in ASD and ID (Moffat et al., 2022).

578

579   To better understand how gene expression varied between genotypes at the different time

580   points, we visualised genes differentially expressed between WT and mutant samples at

581   baseline (home cage test-naive, 0h), 1h and 3h after fear conditioning stimulus. Naive mice

582   showed a small number of DEGs between genotypes (Fig. 5E, 12 DEGs). More DEGs were

583   observed between genotypes in activity-induced transcriptional responses 1h (Fig. 5F, 106

584   DEGs) and 3h (Fig. 5G, 32 DEGs) after training. The majority of DEGs in all conditions were

585   upregulated in the mutant animals, in both female and male mice (Fig. 5E-G), consistent with

586   KDM5B acting as a transcriptional repressor.

587   To understand how the activity-induced transcriptional responses differed between wildtype

588   and mutants, we compared the hippocampal transcriptomes of homecage control mice with

589   mice 1h and 3h post-US. Wildtype animals showed the expected increase in expression of

590   activity-dependent immediate early genes such as *Fosb*, *Npas4* and *Egr3* 1h after training

591   (Fig. 5H, 1h). Three hours after training, gene expression levels of activity genes were back

592   to baseline (Fig. 5H, 3h), consistent with previous studies (Deliu et al., 2018). In addition to

593   the immediate early genes observed in wildtype mice, *Kdm5b*$^{\Delta/\Delta}$ mice displayed a marked

594   upregulation of many more genes (Fig. 5I, 1h; 252 DEGs). Strikingly, three hours after

595   training, >200 genes were still differentially expressed, including immediate early genes like

596   *Fosb*, *Nr4a2* and *Egr3* (Fig. 5I, 3h; 240 DEGs and Extended data Fig. 5-1H-J). Together,

597   these data shows that reduced KDM5B demethylase activity alters the transcriptional

598   landscape in the hippocampus in a number of different ways. These include downregulated

599   baseline levels of immediate early genes, and more pronounced and sustained activity-

600   dependent upregulation following learning relative to these baseline levels. In conclusion,

601   the abnormal expression of several classes of genes implicated in learning and memory in

602   the hippocampus are likely to contribute to the hippocampus-dependent learning deficits in

603   these mice.

604

605

KDM5B directly regulates learning and memory in the adult hippocampus

Our data thus far suggested that KDM5B has a direct role in learning and memory. However, as KDM5B is known to play important roles during development (Catchpole et al., 2011; Albert et al., 2013), the phenotypes observed in $Kdm5b^{\Delta/\Delta}$ mice and other $Kdm5b$-deficient mouse models (Martin et al., 2018; Chen et al., 2023), might all be secondary to abnormal brain development and maturation. To determine if KDM5B has a direct role in learning and memory in the adult brain, we knocked down $Kdm5b$ expression in the dorsal hippocampus (CA1) of adult mice by AAV-mediated delivery of a $Kdm5b$-specific shRNA (Fig. 6A,B).

A quantification of $Kdm5b$ mRNA over time by qRT-PCR, showed a significant knockdown of $Kdm5b$ by 3 weeks after viral delivery, and beginning to return to normal levels by 7 weeks (Fig. 6C). Importantly, we observed a shKDM5B-dependent downregulation of immediate early genes in naïve animals compared to controls that received AAV with a scrambled shRNA (Fig. 6D). This observation suggests that the reduced expression of these immediate early genes in $Kdm5b^{\Delta/\Delta}$ mice (see Fig. 4) are not secondary to developmental abnormalities. $Kdm5b$ knockdown (shRNA) mice showed a significant hyperactivity phenotype in the open field versus controls (Fig. 6E), recapitulating observations with $Kdm5b^{\Delta/\Delta}$ mice (see Fig. 3B). Importantly, $Kdm5b$ knockdown resulted in a substantial deficit in hippocampus-dependent learning and memory in the OLM task (Fig. 6F), similar to those in $Kdm5b^{\Delta/\Delta}$ mice, indicating that $Kdm5b$ is essential for normal long-term memory consolidation in the hippocampus. Exploration time did not show differences between groups in neither training nor testing sessions (Student's t-test: p=0.4940 for training, and p=0.1242 for testing). Furthermore, the shRNA mice exhibited a striking incidence of spontaneous seizures (Fig. 6G), suggesting that $Kdm5b$ knockdown altered neuronal activation and/or circuitry in the hippocampus.

Kdm5b regulates synaptic plasticity

631   To determine if *Kdm5b* knock-down affected synaptic plasticity, we examined short- and

632   long-term potentiation (LTP) in acute hippocampal slices from these mice. We measured

633   LTP in the dorsal CA1b region of the hippocampus, which we have found to be critically

634   important for the hippocampus-dependent object location memory task in previous studies

635   (Barrett et al., 2011; Vogel-Ciernia et al., 2013). Theta burst stimulation produced an

636   immediate increase in potentiation in slices from control mice that gradually decayed to a

637   plateau level that was approximately 50% above pre-TBS baseline (Fig. 6H). Hippocampal

638   slices prepared from *Kdm5b* shRNA mice had similar short-term potentiation to control

639   slices, however, the LTP was significantly less stable as the potentiation fell below control

640   levels 1h post-theta burst stimulation. The mean potentiation for the last 10 min of 1h post-

641   theta burst stimulation was 31 $\pm$ 11% for *Kdm5b* shRNA slices and 47 $\pm$ 6% for control slices

642   (Fig. 6I), suggesting that *Kdm5b* is necessary for the consolidation of LTP in area CA1 of the

643   hippocampus.

644

645   We tested whether synaptic events leading to induction of LTP are negatively affected by

646   *Kdm5b* knock-down by measuring short-term plasticity changes including input/output curves

647   (i/o) and paired-pulse facilitation. The input/output curves were comparable for controls and

648   *Kdm5b* shRNA mice (Fig. 6J). The slope of the io curves was not significantly different

649   between groups ($p<0.90$, data not shown). Paired-pulse facilitation (Fig. 6K), a measure of

650   transmitter release kinetics, also did not show any significant difference between groups and

651   stimulus intervals (2 way-ANOVA; $F_{(2,28)} = 2.28$, $p<0.12$). Thus, it appears that the lack of

652   stability in LTP upon *Kdm5b* knockdown involves cellular events that are set in motion after

653   induction and expression of LTP, an interpretation that is consistent with the observation that

654   short-term potentiation was unaffected in both groups of mice.

655

656

Discussion

Rare coding variants in *KDM5B* are associated with cognitive function in adults and *KDM5B* is associated with a recessive intellectual disability disorder (Faundes et al., 2018; Martin et al., 2018; Chen et al., 2023). In this manuscript, we show that mice homozygous for a hypomorphic allele of *Kdm5b* that lacks H3K4me3 demethylase activity exhibited learning and memory deficits. *Kdm5b* deficiency resulted in alterations in the expression of genes implicated in learning and memory. Acute knockdown experiments showed that *Kdm5b* is critical for normal function of the hippocampus after development has been completed. Notably, two individuals carrying *KDM5B* variants show epileptic spasms and/or generalized seizures (Martin et al., 2018; Mangano et al., 2022), although the latter is more likely associated with a deficit in Nav1. Following *Kdm5b* knockdown in the dorsal hippocampus of adult mice, mice exhibited seizures, consistent with an essential role for *Kdm5b* in maintaining normal circuit homeostasis. These mice had deficits in hippocampus-dependent learning and memory and LTP. Together, this study provides a significant advance in our understanding of the role of KDM5B in learning and memory. Our findings show that KDM5B functions in the adult hippocampus to control learning and memory and imply that at least some of the cognitive deficits associated with KDM5B deficiency could be amenable to treatment in adults.

Learning and memory genes regulated by KDM5B

Our gene expression analyses revealed several potential ways in which KDM5B deficiency might disrupt memory and learning. First, the expression of activity-dependent, immediate early genes was reduced in *Kdm5b*$^{\Delta/\Delta}$ mice. The deletion of several of these genes have been shown to affect long-term memory formation, whilst leaving short-term memory intact (Jones et al., 2001; Fleischmann et al., 2003; Ramamoorthi et al., 2011), similar to our findings with *Kdm5b*$^{\Delta/\Delta}$ mice. Second, we found that these immediate early genes were

28

684   induced by neuronal activation in the hippocampus of *Kdm5b* mutants up to levels equivalent

685   to those in wildtype mice, and therefore hyper-activated relative to baseline levels.

686   Furthermore, these genes were still expressed 3h after neuronal activation in the mutants, at

687   a time point when their expression has returned to baseline in wildtype mice. This inability of

688   gene expression to return to baseline levels is consistent with the H3K4me3 demethylase

689   function of KDM5B. One might speculate that this reduced expression at baseline and

690   apparent over-activation and altered dynamics of IEGs might disrupt normal memory

691   allocation and consolidation mechanisms in *Kdm5b* mutants, theories that need to be tested

692   for *Kdm5b* and other related disorders (Han et al., 2007; Han et al., 2008). For instance,

693   Deliu et al. reported altered trajectories of gene expression in response to conditioning in

694   *Setd5* mutant mice, albeit that baseline gene expression was not markedly different in these

695   mutants compared to wildtype controls (Deliu et al., 2018). Third, *Kdm5b* mutant animals

696   also showed an abnormal overexpression of a cluster of genes that was not affected by

697   neural activity. This cluster, which included proteins with a regulatory role over synaptic

698   function and transcription regulation factors include those genes constitutively affected by

699   the lack of KDM5B demethylase activity. Our results also implicate KDM5B in the regulation

700   of gene transcription through a number of zinc finger proteins, e.g. Zfhx2, Znfx1, Zbtb22 and

701   Zfp810, whose expression is either constitutively affected or abnormally downregulated upon

702   learning in mutant mice (Komine et al., 2012).

703

704   Our GOterms analyses also revealed that WT1 is one of the top transcription factors

705   modulating the expression of genes that are downregulated upon a learning stimulus

706   (Cluster 4, see Fig. 5D). A recent study showed that WT1 regulates neuronal excitability,

707   LTP and long-term memory formation. WT1 limits memory strength, thus enabling the

708   memory flexibility required for normal memory formation (Mariottini et al., 2019). The

709   possible relationship between KDM5B and WT1 warrants further investigation.

710

711   Together, these data showed that the expression of several different types and classes of

712   genes that are regulated by neuronal activation and necessary for learning and memory are

713   affected by *Kdm5b* deficiency.

714

715   **H3K4me3 dysregulation in learning and memory**

716   The finding that mice with *Kdm5b* knockdown in the adult hippocampus exhibit long-term

717   memory deficits, suggests that KDM5B has a direct role in learning and memory,

718   independent from any developmental functions. The most likely mechanism is that a

719   deficiency in KDM5B demethylase activity impacts H3K4me3 dynamics and transcriptional

720   output of key learning-associated genes (Gupta et al., 2010; Collins et al., 2019b; Collins et

721   al., 2019a). Abnormalities may be present in different brain regions and cell types and during

722   distinct stages of memory formation, such as allocation (Han et al., 2007; Han et al., 2008),

723   consolidation (Gupta et al., 2010) and retrieval (Webb et al., 2017). Thus, future studies may

724   need to assess H3K4me3 dynamics at specific gene promoters in different cell types. The

725   conditional deletion of *Kmt2a* and *Kmt2b* in post-mitotic neurons using CamKIIcre was

726   sufficient to cause hippocampus-dependent learning and memory deficits, suggesting a

727   direct role for these genes in excitatory neurons (Kerimoglu et al., 2017). Together, these

728   findings further support the notion that H3K4me3 regulation is important for learning and

729   memory and suggest that dysregulation of H3K4me3, in either direction, can lead to memory

730   deficits. However, as chromatin regulators typically function in large, multi-molecular

731   complexes, other mechanistic explanations cannot be ruled out. Furthermore, a direct

732   comparison between genes regulated by *Kmt2a* and *Kmt2b* has shown that these factors

733   regulate different genes that likely impact neuronal plasticity in distinct ways (Kerimoglu et

734   al., 2017). The two other H3K4me3-specific demethylases, KDM5A and KDM5C have also

735   been implicated in learning and memory. A *Kdm5a$^{-/-}$* mouse model was shown to exhibit

736   deficits in the Morris water maze and *Kdm5c$^{-/y}$* mice have deficits in contextual fear memory

737   and novel object recognition (El Hayek et al., 2020; Vallianatos et al., 2020). Aguilar-Valles

738   *et al.* have shown that *Kdm5c* knock-down in the nucleus accumbens in adult mice results in

739   increased H3K4me3 at gene promoters and deficits in methamphetamine-associated

740   memory formation (Aguilar-Valles et al., 2014). To the best of our knowledge, *Kdm5a* has

741   not yet been deleted or knocked down in post-mitotic neurons in the adult brain, so direct

742   functions for this factor in learning and memory still need to be formally proven. Intriguingly,

743   the contextual fear memory deficits in *Kmt2a*$^{+/-}$ and *Kdm5c*$^{-/y}$ mice were rescued in *Kmt2a*$^{+/-}$

744   ;*Kdm5c*$^{-/y}$ double heterozygous mice, suggesting that these factors regulated the same or

745   similar processes, but in opposite directions (Vallianatos et al., 2020).

746

Cellular/synaptic functions of KDM5B

748   Mouse models for other H3K4me regulators such as *Kmt2a*, *Kdm5c*, *Kdm5a* or *Set1a*

749   display both memory deficits and a decrease in spine density in the hippocampus or cortex

750   (Mukai et al., 2019; El Hayek et al., 2020; Vallianatos et al., 2020). We did not detect

751   significant changes in spine number in the hippocampal stratum oriens and radiatum, or

752   stratum moleculare in the *Kdm5b* mutant mice, suggesting that overt changes in excitatory

753   spine density might not account for the memory deficits in these mutants. The LTP deficit

754   appears to be due to postsynaptic abnormalities, because we did not find any alternations in

755   presynaptic transmitter release. Future studies will need to examine postsynaptic membrane

756   proteins that are known to be involved in LTP and their potential regulation by KDM5B-

757   dependent mechanisms. The exact cell types affected by *Kdm5b* deficiency will also need to

758   be determined.

759

760   We recognise certain limitations in our study. We set out to test if KDM5B demethylase

761   activity have a role in long-term memory consolidation. Although our results are consistent

762   with this hypothesis, KDM5B protein levels were also downregulated in *Kdm5b*$^{\Delta/\Delta}$ mice,

763   limiting our ability to conclusively ascertain the contribution of demethylase deficiency vs.

764   other KDM5B functions. However, it is also intriguing to note that mutations affecting the

765 demethylase activity of *Drosophila* KDM5 leads to memory deficits and decreases

766 neurotransmission (Zamurrad et al., 2018; Belalcazar et al., 2021).

767

768 The relevance of the increased seizures in *Kdm5b* knock-down mice to clinical phenotypes

769 associated with KDM5B deficiency is still unclear. Chen *et al.* recently reported protein

770 truncating variants of *KDM5B* in individuals with epilepsy, but so far, the clinical description

771 of the small number of individuals with recessive *KDM5B* intellectual disability syndrome has

772 not revealed a significant association (Faundes et al., 2018; Martin et al., 2018). We have

773 not observed an obvious increase in spontaneous seizures in our homozygous mouse

774 model. One possibility is that KDM5B deficiency throughout development allows for sufficient

775 homeostatic compensatory mechanisms to ameliorate an overt excitatory/inhibitory

776 imbalance that might lead to spontaneous seizures in some individuals.

777

778 Mutations in chromatin modifying and remodelling factors represent a significant proportion

779 of mutations associated with neurodevelopmental disorders and intellectual disability

780 (Valencia et al., 2023). The functions of most of these factors in the developing and adult

781 brain are still unknown. As these factors are pleiotropic and likely regulates multiple

782 developmental processes and functions in the brain, identifying their salient functions and

783 mechanisms of action remain a significant challenge. This study, together with other recent

784 reports on other chromatin remodelling factors (Kerimoglu et al., 2013; Vogel-Ciernia et al.,

785 2013; Kerimoglu et al., 2017; Chen et al., 2023), suggest that these factors play central roles

786 in the regulation of genes necessary for learning and memory and that intellectual disability

787 disorders and cognitive deficits caused by mutation of these factors may to some extent be

788 caused by direct roles in postmitotic neurons. Reduced function of these factors appears to

789 be associated with altered trajectories of activity-dependent gene expression and abnormal

790 engagement of downstream synaptic plasticity mechanisms (Deliu et al., 2018).

791   Understanding these pathological mechanisms may lead to the development of targeted and

792   improved therapies for intellectual disability disorders.

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798 **Study approval**

799 All animal work were approved by local ethical review panels (AWERB, King's College
800 London) and IACUC (UCI 20-095) and work conducted in the UK approved by a Home
801 Office Project licence (PP6246123).

802

803 **Data availability**

804 RNAseq data (fastq files) were deposited at the Gene Expression Omnibus (GEO) archive
805 under the accession number GSE240887 and made freely available upon publication.

806

807 **Author contributions**

808 MAB, KPG, MAW and LPS conceived the study and designed the experiments and LCA,
809 CF, MAW, KPG and MAB supervised the research. LPS, SB, RM, TG and MAB performed
810 behavioural studies, EK performed LTP experiments, LPS, AG, JAC and AMP performed
811 bioinformatic analyses, FGG, AK and MAB performed surgeries for shRNA knockdown and
812 DPM performed qRT-PCR experiments. LPS, EK and MAB wrote the manuscript with input
813 from co-authors.

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Figures

999 **Figure 1.** *Kdm5b*$^{\Delta/\Delta}$ **mice express reduced total KDM5B levels and show increased**

1000 **number of H3K4me3-positive cells in the hippocampus**

1001 **A)** Schematics of wildtype (WT) and KDM5B$^{\Delta ARID}$ protein domains. The mutant allele results

1002 in a truncated carboxyl end of the JmJN domain (JmjN-T) together with a deletion of the

1003 entire ARID domain. **B)** Representative western blot images from WT (+/+) and homozygous

1004 (Δ/Δ) mutant mice postnatal day 5 (PND5) hippocampal samples showing the lack of full-

1005 length KDM5B protein in cortical samples from Δ/Δ mice. αTubulin was used as loading

1006 control. Full-length KDM5B (arrowhead) and truncated ΔARID (asterisk) protein bands are

1007 indicated. See Extended data Fig. 1-1 for uncropped blot. Molecular weight (M.W.) markers

1008 in kDa are shown on the left. Female (squares) and male (diamonds) samples are included,

1009 although no sex differences were observed. **C, D)** Quantification of full-length and total (full-

1010 length and truncated) KDM5B protein from B. **E, F)** Quantification of the density of

1011 H3K4me3+ (cyan) cells in the hippocampus of 8 week-old wild-type and mutant mice. Nuclei

1012 were counterstained with DAPI (grey). Scale bar, 50μm. Female (squares) and male

1013 (diamonds) samples are included, although no sex effect was observed. **G, I, K)** Cumulative

1014 frequencies of H3K4me3-positive cells as a function of their staining intensity. Cumulative

1015 probability was calculated including all the detected cells (CA1: 406-903 cells, CA3: 236-925

1016 cells, DG: 925-2491 cells; 4 animals/genotype). **H, J, L)** Representative H3K4me3 (cyan)

1017 immunostaining of hippocampal sections in 8 weeks-old control and mutant mice. Sections

1018 were counterstained with DAPI (grey). Scale bar, 200μm. Data in C, D and F is shown as

1019 mean ±SEM and was analysed with Student's t-test. *p<0.05, **p<0.01.

1020 **Figure 2.** *Kdm5b*$^{\Delta/\Delta}$ **homozygous mice are viable but exhibit growth retardation and**

1021 **increased brain:body weight.**

1022 **A)** Homozygous mutants are viable and present at expected Mendelian ratios at P21;

1023 n=128, Chi^2, p=0.9207. Similar Mendelian rates are observed when analysing males and

1024 females separately (Males: 25.38% +/+, 51.52% Δ/Δ, 23.11% Δ/Δ; Females: 27.96% +/+,

1025 47.67% Δ/Δ, 24.37% Δ/Δ). **B)** Pre-weaning body weight (g) measurements show decreased

1026 body weight in mutant animals. **C-F)** Body and brain weight, and brain:body and liver:body

1027 weight ratios are shown for +/+ (n=11) and Δ/Δ (n=13) P21 mice. **G)** Representative images

1028 of brains from wildtype and homozygous mutant mice indicating similar sizes. **H)** General

1029 cortical brain architecture is not affected in homozygous animals. Representative images of

1030 cortical brain sections, where nuclei were visualized with Hoechst3332 are shown. Scale

1031 bar, 50 μm; n=13 +/+ and n=14 Δ/Δ animals. **I)** Layer thickness was measured in the

1032 somatosensory cortex. **J)** Golgi-cox-stained proximal basal and apical dendrites of CA1

1033 pyramidal neurons and apical dendrites of dentate gyrus granule cells in the dorsal

1034 hippocampus were analysed. Graphs depict the average spine density per animal (10-20

1035 dendrites/hippocampal region). Images show representative Golgi-Cox-stained dendrites.

1036 Scale bar, 2.5μm. N=6 mice/genotype. Data is shown as mean ±SEM, including female and

1037 male mice. Data was analysed with two-way ANOVA (B, I) or Student's t-test or Mann-

1038 Whitney test when appropriate(C-F, J). *p<0.05, **p<0.01.

1039 **Figure 3. *Kdm5b*$^{Δ/Δ}$ homozygous mice exhibit hyperlocomotion and learning deficits.**

1040 **A)** Body weight differences in 2 month old mice before the start of the behavioural tests.

1041 Two-way ANOVA genotype effect: ****p<0.0001; sex effect: ***p<0.001. **B)** Distance moved

1042 (cm) in the outer zone of the open field arena. Two-way ANOVA genotype effect:

1043 ***p=0.0005. Three-way ANOVA sex effect: p=0.33. **C)** The amount of time (s) in the inner

1044 zone of the open field is shown. Two-way ANOVA genotype effect: **p=0.0087; sex effect:

1045 *p=0.0107. **D)** Time (%) spent in the open arms in the **e**levated plus maze (EPM), indicative

1046 of reduced anxiety. Two-way ANOVA genotype effect: p=0.8675, sex effect: p=0.4664. **E)**

1047 Discrimination index (DI) during the training and test (24h) phases in the object location

1048 memory (OLM) test to analyse long term spatial memory. Note significant learning in wild-

1049 type mice compared to training, but no significant learning in *Kdm5b*$^{Δ/Δ}$ mutants, and the

1050 reduced DI on test session between the two genotypes. Two-way ANOVA interaction effect:

1051 **p=0.0057. Two-way ANOVA sex effect: p=0.6317 (training) and p=0.9086 (testing) **F)**

1052 There are no genotype differences in total exploration time (s) during training nor testing

1053 session in the OLM test. Two-way ANOVA genotype effect: p=0.2105 (training), p=0.5919

1054 (testing) and sex effect: p=7506 (training) and p=0.2431 (testing). **G)** DI during the testing

1055 session of the spontaneous alternation in a Y maze (Y-SAT), performed 1 h after training to

1056 assess short-term spatial memory. Two-way ANOVA sex effect: p=0.0768. **H)** Spontaneous

1057 alternation rate in the Y-SAT shows no differences between genotypes. **I)** Latency (s) to

1058 reach the hidden platform during the training phase of the Morris water maze (MWM). Data

1059 represents the mean of the 4 trials/day. Two-way ANOVA genotype effect: ***p=0.0001.

1060 Three-way ANOVA sex effect: p=0.0019. **J, K)** Graphs show the number of platform

1061 crossings (J) and time spent (%) in the different quadrants (K) during the probe trial

1062 performed on day 8. Chance, 25%, is depicted with a dashed line. Two-way ANOVA sex

1063 effect in J: p=0.3413. **L)** Swimming speed (cm/s) was similar between genotypes during the

1064 training phase of the MWM. Three-way ANOVA sex effect: p=0.0757, genotype effect:

1065 p=0.1008. **M)** Latency (s) to climb the platform during the visible phase of the MWM. Three-

1066 way ANOVA genotype effect: *p=0.0220; sex effect p=0.1982. **N, O)** Front- and hindlimb grip

1067 strength (gm) was tested three times and the average is shown. Two-way ANOVA sex

1068 effect: p=0.1118 (N) and p=0.1924 (O). **P)** Freezing behaviour was assessed during

1069 contextual fear conditioning training, when three shocks were administered. Three-way

1070 ANOVA sex effect: p=0.07, genotype effect: ****p<0.0001. **Q)** Freezing percentages in the

1071 context testing session, 24 h later. Two-way ANOVA sex effect: p=0.1489. **R)** Mean speed

1072 was higher in mutant mice, in line with their reduced freezing behaviour during training (two-

1073 way ANOVA genotype effect: **p=0.0053). However, no differences were observed between

1074 genotypes during the 2-sec long foot shocks (Tukey's post hoc test p=0.9943 (shock 1),

1075 p>0.9999 (shock 2), p=0.9998 (shock 3), thus discarding differences in sensitivity and

1076 responses to shocks as a contributing factor. Data was analysed with two-way ANOVA (A,

1077 E, F) or repeated-measures two-way ANOVA (B, I, L, M, P, R) followed by Tukey's post-hoc

1078 test. Data in C, J, K, N, O and Q was analysed with Student's t-test, or Mann-Whitney test

1079   when appropriate. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. A one-sample t-test was

1080   used (K) to analyse whether time spent in the target quadrant was above chance. ##p<0.01,

1081   ###p<0.001, ####p<0.0001. For all experiments, n=17 +/+ male, n=14 +/+ female, n=17 Δ/Δ

1082   male and n=13 Δ/Δ female mice. Female (squares) and male (diamonds) samples are

1083   included.

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1085   **Figure 4. Altered gene expression in *Kdm5b*^Δ/Δ^ mice.**

1086   Hippocampi were dissected from naïve animals and RNAseq analyses were performed (n=4

1087   animals/genotype). **A)** Heatmaps show differentially expressed genes in wildtype control

1088   (+/+) and mutant (Δ/Δ) mice. Yellow=upregulated and blue=downregulated in mutants. See

1089   Extended data Table 4-1 for detailed RNA-sequencing data information. **B)** Volcano plot

1090   displaying baseline gene expression changes between wild-type and *Kdm5b*^Δ/Δ^ homozygous

1091   mutant mice. Red and blue dots depict differentially up- and downregulated genes,

1092   respectively. Differentially expressed immediate early genes are labelled. **C)** Immediate early

1093   gene expression in the dorsal hippocampus from WT and *Kdm5b*^Δ/Δ^ mice was analysed by

1094   qPCR; n=9 animals/genotype. Two-way ANOVA genotype effect ***p=0.0001. **D)**

1095   Immunostaining of sections from DG and CA3 with an Npas4-specific antibody (magenta),

1096   counterstained with DAPI (grey) are shown. White arrowheads indicate positive nuclei. **E)**

1097   The number of Npas4-positive cells (arrowheads) was quantified in mm^2 areas of the CA3

1098   and DG. Scale bar, 200μm, 100 μm for higher magnification images; n=10 mice/genotype.

1099   Data is shown as mean ±SEM, including both female and male mice. Data was analysed

1100   with Student's t-test (C, E): *p<0.05, **p<0.01.

1101   **Figure 5. Learning-associated gene expression changes in *Kdm5b*^Δ/Δ^ mice.**

1102   Wildtype (+/+) and *Kdm5b*^Δ/Δ^ (Δ/Δ) mice were trained in the fear conditioning chamber and

1103   culled 1h or 3h later. Homecage, test-naive mice were used as controls. Dorsal

1104   hippocampus was dissected and RNAseq analyses were performed. n=3 males and 3

females per genotype and timepoint. **A-D)** Differentially expressed genes were clustered by their expression trajectories with k means clustering (k=4). Selected genes within each cluster are shown on the right. **E-G)** Heatmaps show differentially expressed genes in control and mutant mice at baseline levels (E) or 1h (F) and 3h (G) after fear conditioning. Yellow = upregulated and blue=downregulated. **H,I)** Volcano plots display gene expression changes after conditioning. Red and blue dots depict differentially up- and downregulated genes, respectively, at 1h or 3h compared to control animals for each genotype. Differentially expressed activity-regulated genes are labelled in orange. Data is shown as mean ±SEM, including female and male mice. Data was analysed with repeated measures two-way ANOVA (A-D): ***$p<0.001$, ****$p<0.0001$. See Fig. 5-1, Table 5-1 and Table 5-2 for extended data.

Figure 6. *Kdm5b* knockdown in the dorsal hippocampus (CA1) of adult mice abrogates hippocampus-dependent memory consolidation and diminishes long-term potentiation.

A) Diagram of experimental work-flow. Approximately 3 weeks after stereotactic viral delivery into CA1, mice were habituated and tested in the object location memory task. Upon completion of behavioural tests, synaptic plasticity was assessed in acute brain slices from these mice. **B)** Representative immunostaining of dorsal hippocampus transduced with GFP-expressing AAV1, 11 days after surgery. Scale bar, 200µm. Panels display representative CA1, CA3 and DG inset images with GFP (green) and DAPI (blue) labelling. Scale bar, 50µm. **C)** qRT-PCR analysis of *Kdm5b* expression, relative to *Hprt*, from total RNA extracted from the dorsal hippocampus at indicated times (3 and 7 weeks (wks) after viral delivery). N=3-5 mice/group. **D)** qRT-PCR analysis of *Kdm5b* and immediate early gene expression, relative to *Hprt*, from total RNA extracted from the dorsal hippocampus, 11 days post-transduction. Two-way ANOVA shRNA effect for IEGs: **$p=0.0016$. N=3 mice/group. **E)** Distance moved (cm) in the test arena (open field) on the first day of habituation as a

45

1132   measure of general activity (n=10 control and n=10 shRNA females). **F)** Discrimination index

1133   in the OLM task for control and shRNA mice during training and 24h long-term memory tests

1134   are shown. Note significant learning in control mice compared to training, but no significant

1135   learning in shRNA mice compared to controls (n=9 control and n=9 shRNA female mice).

1136   Two-way ANOVA interaction effect: *p=0.0257. **G)** Number of mice of each group (n=10

1137   each) showing spontaneous seizures during handling (pink), compared to no seizures (grey).

1138   Fisher's exact test: **p=0.0031. **H)** Short- and long-term plasticity changes measured from

1139   hippocampal area CA1b apical dendrites in acute hippocampal slices (n=8 slices from each

1140   group; n=4 mice/group). Following a 20 min stable baseline recording, theta burst stimulation

1141   (TBS, arrow) was delivered to induce LTP and recordings were followed for an additional 1h.

1142   The fEPSP slope measured from *Kdm5b* shRNA slices was noticeably lower relative to

1143   controls by the end of the recording period. Inset: representative traces collected during

1144   baseline (black line) and 60 min post-TBS (red line). Scale: 1 mV/5 ms. **I)** The mean

1145   potentiation 50-60 min post-TBS was significantly reduced in slices from *Kdm5b* shRNA

1146   mice relative to controls (**p = 0.0056, n=8 each). **J, K)** Short-term plasticity measures in

1147   slices from *Kdm5b* shRNA mice including (J) input/output curve and (K) paired-pulse

1148   facilitation did not reveal any significant differences from shScramble controls. Data is shown

1149   as mean ±SEM, analysed with Student's T-test (C, D, E, I), two-way ANOVA (F) and Fisher's

1150   exact test (G). *p<0.05, **p<0.01, ***p<0.001. Control, shScramble; shRNA, shKdm5b.

1151

1152

1153

A

+/+
27.1%

Δ/Δ
23.8%

+/Δ
49.1%

B

+/+
Δ/Δ

**

Weight (g)

P3 P6 P9 P12 P21
Postnatal day

C

Body weight (g)

*

+/+ Δ/Δ

D

Brain weight (g)

+/+ Δ/Δ

E

Normalized brain/body weight

**

+/+ Δ/Δ

F

Normalized liver/body weight

+/+ Δ/Δ

G

+/+   Δ/Δ

1 cm

H

+/+   Δ/Δ

I
II/III
IV
V
VIa
VIb

I

+/+
Δ/Δ

Layer thickness (μm)

I II/III IV V VIa VIb

J

S. Radiatum   S. Oriens   S. Moleculare

Spine density (spines/μm)

+/+ Δ/Δ   +/+ Δ/Δ   +/+ Δ/Δ

+/+   +/+   +/+
Δ/Δ   Δ/Δ   Δ/Δ

A +/+ Δ/Δ
♀ ♂ ♀ ♂

B Δ/Δ vs. +/+

-Log$_{10}$(FDR)

Egr1

Npas4

log2(FC)

C

☐ +/+ ■ Δ/Δ

Fold change

Egr1 Npas4 cFos Egr2 Arc

* * *

D

+/+ Δ/Δ

DG

Npas4
DAPI
Merge

CA3

Npas4
DAPI
Merge

E

Npas4+ cells/mm^2 (%)

**

+/+ Δ/Δ

A

+/+
Δ/Δ

Npas4
Egr1
Nr4a2
Fosb
Dusp6
Homer1
Kdm6b
Per1

Cluster 1

B

+/+
Δ/Δ

Bcl6
Neurod2
Foxo3
Fgfr1
Notch4
Disp3
Sorl1
Xdh

Cluster 2

C

+/+
Δ/Δ   ****

Atf5
Stat6
Ago2
Stat4
Foxn3
Bhlhe40
Pura
Zfp456

Cluster 3

D

+/+
Δ/Δ   ***

Sox9
Pde10a
Hgf
Tgfb2
Atf6
Rorb
Usf2
Zfp974

Cluster 4

E Baseline   **F** 1h post-CFC   **G** 3h post-CFC

♂
+/+
♀

♂
Δ/Δ
♀

H   +/+

1h   3h

I   Δ/Δ

1h   3h

A

AAV-Kdm5bshRNA or
AAV-scrRNA

3 weeks

B

GFP DAPI

CA1

DG

CA3

CA1   CA3   DG

GFP

DAPI

Merge

C

Relative *Kdm5b* expression

***   ns

Ctrl-3wks   shRNA-3wks   Ctrl-7wks   shRNA-7wks

D

Control   shRNA

Fold change

**

Kdm5b

Fold change

*

Arc   cFos   Egr1   Egr2   Npas4

E

Distance moved (m)

*

Control   shRNA

F

Discrimination index (%)

ns

***   **

Control   shRNA

Training   Testing

G

Number of mice

No seizures   Seizures

**

Control   shRNA

H

fEPSP slope (% baseline)

Kdm5b shRNA
Control

shRNA   Control

TBS

minutes

I

Mean Potentiation
50-60 min post-TBS

**

Control   shRNA

J

fEPSP slope (mV/ms)

shRNA   Control

K

PPF (%)

shRNA   Control

400   100   200